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Communication to the Editor

Distribution of Sensitivity Responses to Cymoxanil within Global Populations of *Phytophthora infestans*†

Ronald A. Hamlen* & Richard J. Power

E. I. DuPont de Nemours and Company, Agricultural Products, Stine-Haskell Crop Research Center, Newark, DE 19714-0030, USA

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Abstract: In-vitro and in-vivo results show no decrease in *Phytophthora infestans* sensitivity to cymoxanil after more than 16 years of intensive use, and that a single, sensitive population exists within the countries sampled. © 1998 SCI

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1 INTRODUCTION

Cymoxanil-based fungicides are used worldwide to control downy mildew diseases on more than 15 crops grown on over 10 million hectares. They are key materials for controlling the fungal pathogen, *Phytophthora infestans* (Mont.) de Bary the cause of potato and tomato late blight—the disease responsible for the Irish potato famine of 1845–1849. While cymoxanil was introduced 20 years ago, its toxicological and environmental profile, and its efficacy against emerging fungicide-resistant and increasingly aggressive and genetically diverse strains of *P. infestans*, sustain its value for the twenty-first century. Maintenance of marketplace confidence in product performance, as well as good product stewardship, demand a clear understanding of the sensitivity of contemporary *P. infestans* populations to cymoxanil.

2 EXPERIMENTAL METHODS

A world-wide collection of 238 isolates of *P. infestans* was tested for sensitivity to cymoxanil by an *in-vitro* radial growth assay.[1] Isolates obtained from single lesions or single zoospores were, for the most part, genetically different by mating type, host, alleles at allozyme loci (*Gpi* and *Pep*), restriction fragment polymorphism (RFLP), and they were geographically diverse. Fresh collections were assayed whenever possible, limited sub-culturing was carried out and, when necessary, cryo-storage (liquid nitrogen) of single-spore isolates was used to maintain isolate integrity. Assay precision and consistency were validated by single-zoospore reference isolates' responses over a several-month assay interval. A greenhouse study was used to evaluate cymoxanil's efficacy (as the commercial

* To whom correspondence should be addressed.

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500 g kg^{-1} WP Curzate®) against isolates with differing in-vitro sensitivities.

3 RESULTS AND DISCUSSION

Variation in sensitivity levels among 191 *P. infestans* isolates collected in 1980–1996 from populations in France, Germany, Ireland, Italy, Mexico, Poland, Spain, Switzerland, The Netherlands, the UK and the US which had been exposed to cymoxanil for up to 16 years was low, with EC_{50} values ranging from 0·06 to 1·48 mg litre^{-1} (Fig. 1). The sensitivity responses of isolates collected from these contemporary populations were not extensively different from those of isolates collected prior to cymoxanil commercialization. The means ($\pm$SD) and variance of the EC_{50} values for pre-commercialization (47 isolates) and contemporary populations were 0·19 ($\pm$0·12) and 0·01 mg litre^{-1} and 0·42 ($\pm$0·25) and 0·06 mg litre^{-1}, respectively, and represent a negligible 2·2-fold difference in mean response.

Examining the distribution of responses among *P. infestans* isolates from Europe, Mexico and the US when sorted by collection year (1980–1996) indicated that year-to-year differences were small and random in response to cymoxanil, i.e. yearly mean EC_{50} values ranged from 0·25 to 0·51 mg litre^{-1}. The mean EC_{50} value for isolates from a pre-commercialization population in Germany, the UK, and The Netherlands was 0·22 mg litre^{-1}, compared with 0·46 mg litre^{-1} for the response of 64 contemporary isolates where 88% of the individuals came from populations which had been intensively and extensively exposed to cymoxanil for 10 or more years. A US population exposed for only one to two years and a US pre-commercialization population exhibited comparable mean EC_{50} values of 0·50 and 0·22 mg litre^{-1}, respectively. These data might indicate that small initial shifts in sensitivity occurred following cymoxanil commercialization, or possibly reflect a greater population variation due to the replacement of established populations by new genotypes.[2,3] With only a 2·1 to 2·3-fold difference in mean EC_{50} response between contemporary and pre-commercialization populations with widely different exposure histories, it appears that differences between contemporary and pre-commercialization populations, as well as among individual years, are small and inconsequential. Most significantly, no correlation ($r^2 = 0{\cdot}005$) between isolate sensitivity and years of cymoxanil exposure was observed, i.e. no decrease in sensitivity level was found and variation in EC_{50} values was not explained by years of exposure (Fig. 2).

While *in-vitro* differences in sensitivity were observed in this study, it is well known that such *in-vitro* assays can measure minute differences that are not observable in whole-plant tests, or in the field.[4] To investigate this phenomenon a least-sensitive A1 mating type isolate (high *in-vitro* EC_{50}) from the contemporary population and a most-sensitive pre-commercialization A2 type isolate (low EC_{50}) were inoculated onto greenhouse-grown potatoes (cv. Bintje) treated with cymoxanil 500 g kg^{-1} WP. Mean EC_{50} values for the pre-commercialization and contemporary isolates were 40 and 23 g ha^{-1}, respectively. The results confirmed the efficacy of cymoxanil against *P. infestans* isolates with widely diverse EC_{50} values as observed in these *in-vitro* assays. Control of both isolates was achieved with the field rate of 100 g ha^{-1} cymoxanil.

Equivalent activity was also observed against 84 *P. infestans* isolates with known mating type, including isolates of different genotypic structure recently immigrated from Mexico to the US, and perhaps worldwide.[2,5] The mean EC_{50} values for the A1 and A2 mating types were 0·33 and 0·35 mg litre^{-1}, respec-

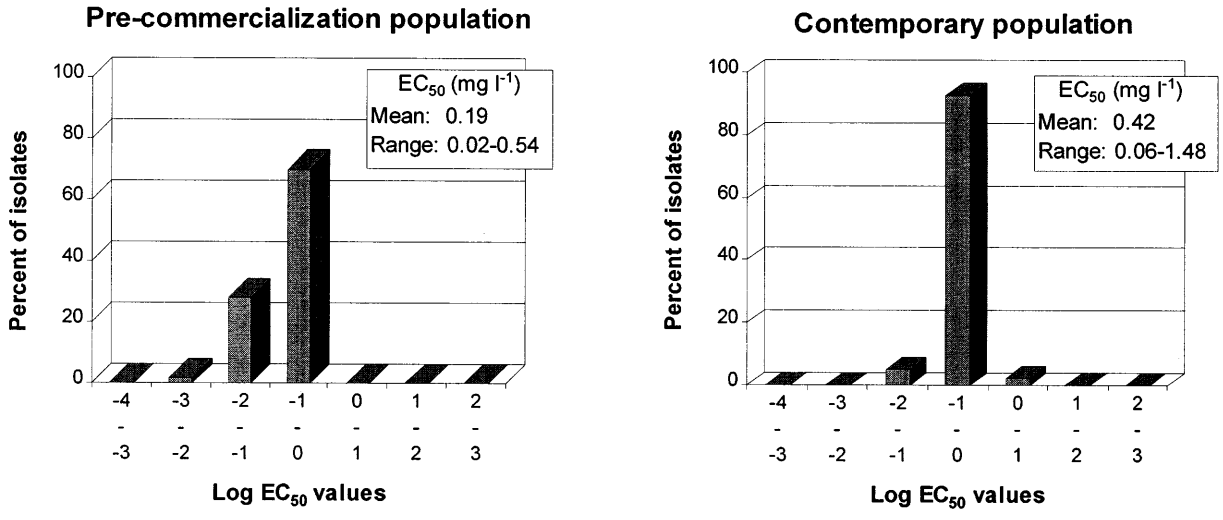

Fig. 1. Variation in sensitivity responses to cymoxanil among *Phytophthora infestans* isolates collected in 1980–1996 from populations in France, Germany, Ireland, Italy, Mexico, Poland, Spain, Switzerland, The Netherlands, UK and US exposed to cymoxanil for up to 16 years, compared to responses of isolates from pre-commercialization populations.

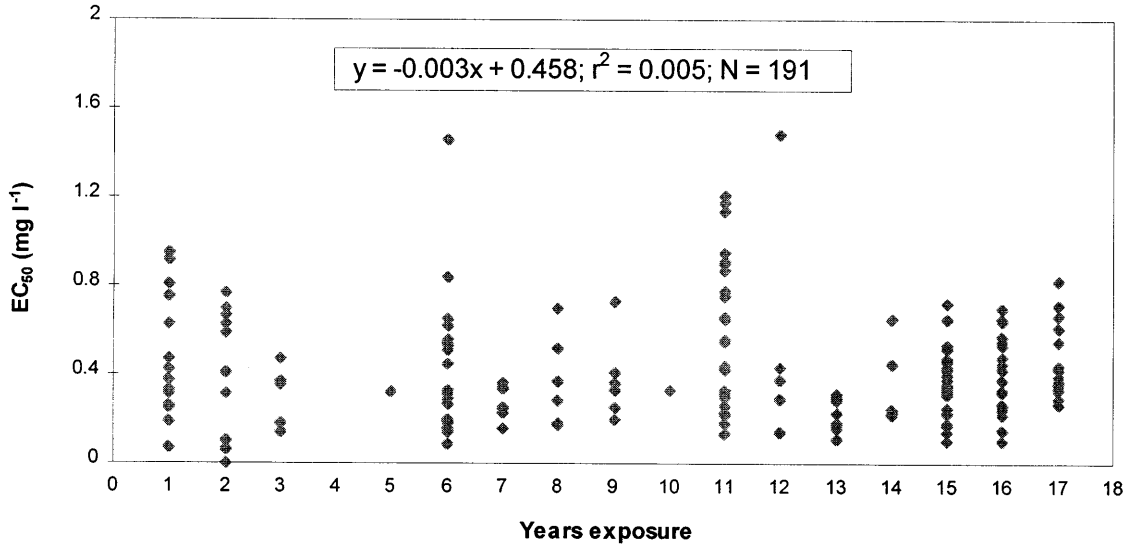

Fig. 2. Sensitivity of *Phytophthora infestans* isolates relative to years of exposure to cymoxanil. Data are plotted as a scatter diagram comparing exposure, measured as time from the year of commercialization to the year of isolate collection, *versus* individual isolate EC_{50} values.

tively. These data are consistent with previous results with a collection of isolates from central and NW Mexico.[6] Adequate control of both mating types is essential, as the single clonal lineage know to exist worldwide for *c.* 100 years has been replaced by immigrant populations with different genotypic structure.[3] These new populations consist of A1 and A2 types, creating the possibility of genetic recombination and production of new strains with different pathogenic and/or fungicide sensitivity characteristics.

4 CONCLUSIONS

Based on these results we conclude that the variation in sensitivity phenotype within a genetically diverse and contemporary worldwide population of *P. infestans* is low. Moreover, there has been no decrease in sensitivity to cymoxanil after more than 16 years of intensive use, and a single, sensitive *P. infestans* population exists within the sampled countries that is effectively controlled by cymoxanil-based fungicides used according to label recommendations. These conclusions are supported by the multi-site mechanism of action of cymoxanil-based fungicides, use of cymoxanil in mixtures in preventive application schedules, the failure to induce laboratory mutants of *Phytophthora* spp. resistant to cymoxanil (Leroux, P., 1981, pers. comm.), and by no evidence of field control failures of *P. infestans*. The worldwide sensitivity characteristics of *P. infestans* populations will be assessed further to maintain our awareness of cymoxanil efficacy against this destructive and genetically dynamic pathogen.

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